



Comparative efficacy of *Beauveria bassiana*, *Bacillus thuringiensis*, and aldicarb for control of Colorado potato beetle in an irrigated desert agroecosystem and their effects on biodiversity

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Abstract

Five weekly applications of *Beauveria bassiana* (Balsamo) Vuillemin, a genetically engineered isolate of *Bacillus thuringiensis* Berliner (Raven®), and aldicarb (Temik®) were compared for control of Colorado potato beetle, *Leptinotarsa decemlineata* (Say) in an irrigated desert cropping system. *B. thuringiensis* was applied using low and high label rates (1.17 and 7.0 l ha⁻¹). *B. bassiana* was applied at 5×10^{13} spores ha⁻¹. Aldicarb (Rhône-Poulenc), applied at 3.37 kg a.i. ha⁻¹ provided the greatest beetle control and potato yields (45 metric tons ha⁻¹), but overall lowest biodiversity in nontarget organisms, particularly predatory Heteroptera. Low and high rates of *B. thuringiensis* produced fair to excellent beetle control, yielded 33 and 40 metric tons ha⁻¹ and enabled good survival in predatory Heteroptera and other nontargets. Plots treated with *B. bassiana* resulted in poor control of beetles prior to row closure after which fair to good control was achieved. Yield in the *Beauveria*-treated plots was 33 metric tons ha⁻¹ and effect on biodiversity was comparable to the *Bt*-treated plots. The lowest number of overwintering adult *L. decemlineata* was found in the plots treated with bacteria and fungi (0.68–0.84 adults/0.03 m⁻³ of soil) and the highest was found in control and aldicarb plots (3.44 and 1.84 adults/0.03 m³ of soil). Aphids and leafhoppers showed higher densities in plots treated with microbial control agents, but were eliminated from plots treated with aldicarb.

Introduction

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), is one of the most serious insect pests of potato in North America and Europe (Casagrande, 1987; Hare, 1990). Traditional interventions include treatment with a range of broad spectrum insecticides such as carbofuran, fenvalerate and aldicarb (Temik®). Reliance on conventional insecticides has resulted in multiple resistance in CPB (Casagrande, 1987; Hare, 1990) and a variety of untoward effects on nontarget organisms and the environment. Aldicarb, for example, has high toxicity for vertebrates and other nontarget organisms (Brown, 1978).

An integrated control strategy that makes maximum use of ecologically sound interventions will

provide a number of benefits including management of insecticide resistance and reduction of pesticide use, and will contribute to a more sustainable and environmentally friendly approach to CPB management (Casagrande, 1987; Boiteau et al., 1995). A major component of IPM for control of CPB would be the concerted use of biological control (Ferro, 1994; Cloutier et al., 1995). Biological control agents of CPB include a variety of predatory insects, parasitoids and microbial control agents.

The fungus, *Beauveria bassiana* (Balsamo) Vuillemin and beetle-active isolates of the bacterium, *Bacillus thuringiensis* Berliner var. *tenebrionis* Kreig are two entomopathogens with good potential for control of CPB (Cloutier et al., 1995). The majority of published research on microbial control of CPB in the

United States is from the Eastern half of the country where a significant portion of potato production is rainfed and higher humidities prevail. However, more than 54% of potato production is from the much drier northwestern states of Idaho, Washington, and Oregon (USDA-NASS, 1998). Field efficacy studies of microbial control agents of CPB in irrigated desert agroecosystems of the Pacific Northwest have been somewhat limited. In this paper we report the results of comparative field trials of *B. bassiana*, *B. thuringiensis* and aldicarb for control of CPB and their effects on beneficial and other nontarget insects in an irrigated desert cropping system.

Methods and materials

Experimental design. Five treatments were set out in a complete randomized block design having five replicates/treatment. Plots were located at an experimental research farm located just east of Yakima, WA. Each plot was 15 m long \times 5 rows wide. Adjacent rows within a plot were separated by 0.85 m. Seed potato tubers (Russet Burbank) were planted on 9 May 1997 at 0.3 m spacings to produce a density of 250 plants plot⁻¹ (5 rows \times 50 plants row⁻¹). Adjacent plots were separated by a 3 m strip of Sudan grass [*Sorghum sudanense* (Piper)], which acted to reduce movement by adult beetles between plots. Plots were irrigated with sprinklers at ca. weekly intervals for 6.5 h resulting in 4.4 cm of water per unit area. Irrigation was applied the day before application of microbial control agents.

Overwintered beetles were collected in early spring from volunteer potato plants growing in a different area of the farm and were released in the experimental plots on 10 June 1997. Beetles were released at the rate of 250–300/plot (1–1.2/plant) to produce similar starting densities among plots (natural infestation rates were low). A pre-treatment census of adults, larvae, and egg masses was made on 16 June 1997 to confirm that initial densities were similar among plots.

Treatments. An oil flowable formulation of *B. thuringiensis* (*Bt*) (Raven® batch ORV 513704, Ecogen, Langhorn, PA, USA) was used at specified low and high label rates of 1.17 l ha⁻¹ and 7.0 l ha⁻¹ on each of five plots. The Raven product is made with a strain of *B. thuringiensis* var. *kurstaki* (EG 7673) that has been genetically modified using recombinant DNA technology to overproduce a unique coleopteran-active cry

3 Bb toxin (Baum et al., 1996). The product label indicates that the percentage of beetle-active toxin is 8.0%. The Mycotech WP formulation of *B. bassiana* (Mycotrol WP lot # 970429-1, Mycotech, Butte, MT, USA) was applied to 5 plots at the recommended rate of 5×10^{13} conidia ha⁻¹. Manufacturer-determined viability rate of 93% was confirmed with germination tests on Sabouraud dextrose agar as prescribed by Goettel & Inglis (1997). The conventional insecticide treatment consisted of aldicarb applied at the rate of 3.37 kg [a.i.] ha⁻¹. Aldicarb granules (15% a.i.; Temik 15G, Rhône-Poulenc Agro, Lyon, France) were applied as a side dressing (10 cm deep, on each side of potato rows) using a Gandy model 901-4 granule applicator on 30 May 1997 when potatoes plants were emerging.

For treatment of individual plots with microbial control agents, the desired amount of each agent was thoroughly suspended in 5.9 l of deionized water and applied using an R & D Model T (R & D Sprayers, Opelousas, LA, USA) CO₂ powered sprayer at 276 kPa (40 psi). The sprayer was equipped with a hand held spray boom configured with two sets of five nozzles to provide coverage of two rows at a time. Each five nozzle array (three 80 °C *t*-jet nozzles and two 110 °C *t*-jet nozzles/plant/row) ensured complete coverage of foliage from the top, sides and underside of the plants just to the point of runoff. Control plots received 5.9 l of water. Five applications were made at weekly intervals commencing on June 26 when plants were just under 0.6 m high and ending July 22. Applications were made between 18:30 h and 20:30 h to minimize solar inactivation of pathogens.

Sampling CPB. Densities of all stages of CPB were estimated by taking whole-plant counts. Ten plants plot⁻¹ were sampled at biweekly intervals beginning 10 d before the initial application of pathogens (16 June) and ending in mid-August once control plots had been completely defoliated by CPB. The ten plants in each plot were selected randomly from the interior three rows of each plot. Each plant was carefully examined for four life stages of the beetle: egg masses; small larvae (instars I–II); large larvae (instars III–IV); and, adults.

Defoliation estimates were made visually in each plot 7 d following the 4th treatment date and again 7 and 21 days following the 5th and final application of microbials using the index system of Sinden et al. (1986). Damage was scored on a 0 to 5 scale: 0, no feeding; 1, a few leaflets partially eaten; 2,

some leaflets completely eaten; 3, at least one stem defoliated; 4, all stems nearly defoliated; 5, complete defoliation.

Sampling of live and dead CPB and percentage of fungal infection in beetles/929 cm² (1 ft²) of soil surface in plots treated with *B. bassiana* or with the low rate of *B. thuringiensis* was conducted on August 7. Plots that had been treated with the low rate of *B. thuringiensis* were used as a comparison to reveal background infections and because they were similarly infested and had identical defoliation ratings as the *B. bassiana* plots. Control plots were unsuitable for sampling due to severe defoliation, and plots treated with the high rate of *B. thuringiensis* and aldicarb were nearly devoid of larvae. At each of five sampling sites within each plot, two paired 929 cm² samples were taken. One sample on the hill and one in the furrow were taken 2 m into the plots at each end of the furrows between rows 2 and 3 and rows 3 and 4 and in the middle of the plot.

Sampling for overwintering beetles was conducted on October 7 after vines in all plots had senesced. Five 0.03 m³ (1 ft³) soil samples (31 cm × 61 cm × 15 cm deep) were taken with a spade 2 m into the plots at each end of the furrows between rows 2 and 3 and rows 3 and 4 and in the middle of the plot. The soil samples were sieved through a 0.64 cm mesh screen and living, dead and mycosed adults were counted.

Sampling of nontarget arthropods. Densities of predatory and phytophagous insects occurring on plants were determined using beating trays. Trays (46 cm × 46 cm) were held beneath plants and the plants were vigorously shaken to dislodge arthropods. Specimens were aspirated from the trays into tubes using battery-operated aspirators, and the contents of each tube were then transferred to a vial of alcohol for later processing. We sampled 10 random plants from the interior 3 rows of each plot. Sampling occurred at biweekly intervals beginning with the first sample on 2 July, 1997.

Pitfall traps were used to sample ground-dwelling arthropods. Traps were composed of plastic drinking cups (0.3 l) placed in the ground such that the rim of the cup was flush with the soil surface. Cups were then filled ca. half-way with ethylene glycol. Three traps plot⁻¹ were set out in the center row of each plot. Distance between adjacent traps or between traps and the plot edge was ca. 3.8 m. Traps were set out on 2 June 1997 and sampled at biweekly intervals.

Our objective in this portion of the study was not to fully survey all arthropods associated with the different treatments, but to determine the effects of the treatments on a few select groups of arthropods (specifically, natural enemies, potential prey for natural enemies, and potato pests). Thus, for the most part, arthropods collected using either trays or pitfall traps were categorized only very broadly (i.e., to Order or Family). Some taxa of more specific interest [e.g., *Geocoris* spp.; *Orius tristicolor* (White)] were identified to species or genus.

Tuber yield. Potatoes were harvested on October 28 from the center three rows of each plot using a tractor drawn single row harvester that had been modified for sorting out unwanted materials (rocks, dirt clods, etc.) and sacking of marketable tubers. The sacks were weighed in the field with a Pelouze Model P1005 heavy duty scale.

Statistical analyses. Densities of CPB were compared among treatments using analysis of variance (ANOVA). Plots were sampled repeatedly through time, so an initial test was done using a repeated measures ANOVA to determine the significance of the sampling date × treatment interaction. The interaction was invariably significant, indicating that magnitude of treatment effects depended upon date of the sample, thus the repeated measures analyses were followed by one-way ANOVA (with four treatments and one control) conducted separately for each date. If the one-way ANOVA was significant, treatment means were separated using the LSD test. This same statistical approach was used to determine the effects of treatment on densities of arthropods collected using the beating trays or pitfall traps. Plot means, rather than individual plant counts, were used in all analyses. Analyses were done in PROC GLM (SAS Institute, 1996).

Defoliation ratings were compared among treatments using a Friedman's test followed by a non-parametric version of the LSD test (Conover, 1980). Analyses were done using PROC RANK and PROC GLM (SAS Institute, 1996). Effects of pathogen type (*B. thuringiensis* vs *B. bassiana*) on densities of beetle cadavers at the base of plants were determined using ANOVA. Two paired locations at each of five sites were sampled in each plot (furrow vs hill), and location effects were included in the ANOVA as a repeated-measure factor. Effects of treatment on the number of overwintering beetles on densities of beetle cadavers were determined using ANOVA. Tu-

ber yields were compared among treatments using ANOVA. Treatment means were separated using the LSD test.

Results

Beetle densities and plant damage

The number of egg masses/plant before application of the biopesticides was similar in control plots and those to be treated with *B. thuringiensis* (3.43–3.93/plant), but significantly higher in the plots to be treated with *B. bassiana* (5.21/plant; $F=6.08$, $df=3,12$, $P=0.009$). The pretreatment numbers of small larvae and adults were not significantly different between control plots and those that were to receive biopesticide treatments (small larvae: $F=1.35$; $df=3,12$; $P=0.30$ and adults: $F=0.39$; $df=3,12$; $P=0.76$). No eggs, larvae or adults were found in the June 16 sampling of the aldicarb-treated plots (Figure 1).

Bi-weekly sampling up to and including July 29 revealed a distinct developmental progression of the CPB first generation cohort in control plots (Figure 1). After July 29, control plot plants were nearly defoliated and second generation adult beetles began invading adjacent plots.

Following the first application of biopesticides on June 26, sampling July 1 revealed near complete and complete protection in the plots treated at the high rate of *Bt* and aldicarb, respectively. Egg masses were significantly more abundant in the control, low *Bt*, and fungus-treated plots than in aldicarb-treated plots ($F=7.39$; $df=4,16$; $P<0.001$; LSD-test used to separate treatment means). Control and low *Bt* plots had significantly higher numbers of larvae (combined) than high *Bt* and aldicarb plots, but significantly lower numbers than fungus-treated plots ($F=31.18$; $df=4,16$; $P<0.001$) (Figure 1). The third sampling for CPB (July 15) took place after the 2nd and 3rd applications of microbials (July 2 and 8). Peak numbers of larger larvae and a general decline in numbers of small larvae were observed in this sample with a distinct high level of protection in the high rate *Bt* and aldicarb plots. Significantly fewer large larvae were observed in the low *Bt* and fungus-treated plots relative to controls, but significantly more than that of the high *Bt* and aldicarb plots ($F=42.02$; $df=4,16$; $P<0.001$). Numbers of small larvae/plant were comparable in the controls and fungus-treated plots, and both treatments were significantly more highly infested than the low *Bt* plots ($F=17.02$; $df=4,16$; $P<0.001$) (Figure 1).

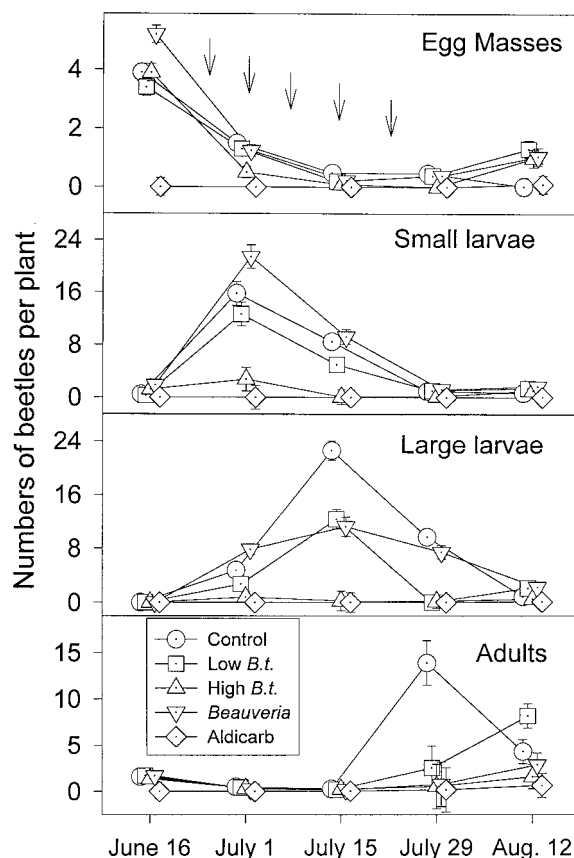


Figure 1. Mean (\pm s.e.) numbers of Colorado potato beetle per plant in experimental plots. Post-treatment (1 July to 12 August) counts compared among treatments by one-way ANOVA conducted separately for each date. Least significant differences for sample dates on which the ANOVA was significant are: egg masses per plant (1 July to 12 August, respectively) 0.69, 0.25, 0.36, and 0.88; small larvae per plant (1 July to 29 July, respectively) 5.29, 3.22, and 0.60; large larvae per plant (1 July to 12 August, respectively) 2.51, 4.38, 2.86, and 1.46; adults per plant (1 July, 29 July, and 12 August, respectively) 0.26, 7.05, and 3.83. Arrows depict application dates for pathogens.

Defoliation ratings in Table 1 on July 22 in the different treatments were ranked similarly to mean densities of large larvae in the July 15 sample (Figure 1). The least damage was observed in the high *Bt* and aldicarb plots and increasingly greater defoliation was observed in the low *Bt*, fungus-treated and control plots respectively (Table 1). On this sampling date, fungus-infected cadavers were noticeable for the first time. This was coincidental with row closure. Also the 3rd application of *B. bassiana* coincided with a weather front that resulted in considerable cloud cover, but negligible amounts of rain.

Table 1. Defoliation rating in plots receiving no interventions for Colorado potato beetle and those treated with the Mycotrol WP formulation of *Beauveria bassiana*, the Raven flowable formulation of *Bacillus thuringiensis* or aldicarb

Treatment	Mean defoliation rating ^a (median defoliation rating)		
	July 22 ^b	August 5 ^c	August 19 ^d
Control (no intervention)	3.0 a (3)	4.0 a (4)	5.0 a (5)
<i>B. bassiana</i> (5×10^{13} spores ha ⁻¹)	2.0 b (2)	1.2 b (1)	2.8 c (3)
<i>B. thuringiensis</i> (1.17 l ha ⁻¹)	1.4 c (1)	1.2 b (1)	3.6 b (3)
<i>B. thuringiensis</i> (7.04 l ha ⁻¹)	0 d (0)	0 c (0)	2.4 d (2)
aldicarb (3.37 kg a.i. ha ⁻¹)	0 d (0)	0 c (0)	0.2 e (0)

^aUsing the defoliation rating system of Sinden et al. (1986); defoliation means followed by the same letter in the same column are not significantly different from one another as determined with Friedman's ANOVA and a nonparametric multiple range test (Conover, 1980).

^b7 d after 4th application (F=306.00; df=4,16; P<0.001).

^c14 d after 5th and final application (F=51.63; df=4,16; P<0.001).

^d28 d after final application (F=74.33, df=4,16 P<0.001).

The 4th sampling date (July 29), followed the penultimate and final applications of microbial control agents (July 15 and 22) and revealed heavy populations of adults in control plots (13.9/plant), moderate numbers of large larvae (9.7 and 7.4/plant, respectively) in control and fungus-treated plots and significantly lower numbers of larvae in the *Bt* and aldicarb-treated plots (F=20.66; df=4,16; P<0.001).

Samples of live, dead and fungus-infected beetles on the soil surface in *B. bassiana* and low *Bt*-treated plots were taken 16 d after the final treatments (August 7) and revealed significant differences between the two treatments and between the two sampling locations (Table 2). Significantly more dead and fungal infected larvae were found on the surface of the ground in the *B. bassiana*-treated plots than in *Bt*-treated plots. Also significantly greater numbers of insects were found in furrows than on hills. Most of the cadavers in both treatments were fungal infected larvae. The presence of fungal infections in the *Bt* plots apparently reflects the natural occurrence of *B. bassiana*. It is highly probable that many of the *Bt*-killed larvae, especially young larvae that had died following application of

the microbials were not detected due to their size and decomposition.

The fifth and final sample of CPB (August 12), taken 19 d after the final application of biopesticides, showed a sharp decline in adult beetles in the entirely defoliated control plots and an increase in adult beetles in all of the treated plots, ostensibly due to immigration. Numbers of adult beetles/plant were significantly higher in the low *Bt* plots than in the controls and all other treatments (F=5.33; df=4,16 P<0.01). Although immigration from control plots was a likely source, adult beetles may also have originated from within these plots due to retarded development of surviving larvae. The numbers of large larvae were significantly greater in the low *Bt* and fungus-treated plots compared to high *Bt* and aldicarb treatments (F=4.13; df=4,16 P=0.02). Defoliation samples taken the following week (August 19) demonstrated only slight damage in the aldicarb-treated plots and significant damage in all of the biopesticide-treated plots (Table 1).

Effect of treatments on overwintering beetles. Overwintering beetles were at highest densities in control plots despite the massive emigration to other plots following defoliation. (Table 3). The next highest densities of living beetles were found in aldicarb-treated plots. This is probably due to the fact that when all other plots were defoliated the aldicarb-treated plots offered the only local source of undamaged potato vegetation. Numbers of living adult beetles in the plots treated with microbials were relatively low (0.68–0.84 beetles/0.03 m³) and comparable for the three treatments. Significantly higher numbers of mycosed cadavers, mostly larvae, were found in the fungus-treated plots, but some mycosed individuals were also found in each of the other treatments and controls.

Yield. Potato yields were significantly lower in control plots compared to all other treatments (Table 4). Plots treated with *Bt* at 1.17 l ha⁻¹ and *B. bassiana*-treated plots yielded identical tonnage of potatoes and significantly less than the 7.04 l ha⁻¹ *Bt* treatment. The highest yield was obtained in aldicarb-treated plots. Yield for the various treatments and defoliation scores on August 19 tended to be inversely related (Tables 1 and 4). There were significant block effects associated with the yield data (F=5.4, df=4,16, P<0.01), which may reflect either variations among blocks due to soil fertility or amount of water, or variation due to differential immigration by beetles.

Table 2. Average number of live and dead Colorado potato beetles and percentage of fungal infection in cadavers per 929 cm² (ft²) of soil surface in plots treated with the Mycotrol WP formulation of *Beauveria bassiana* (5×10^{13} conidia ha⁻¹) or with the Raven flowable formulation of *Bacillus thuringiensis* (1.17 l ha⁻¹)

Treatment	Mean no. \pm s.e. of beetles/929 cm ² (% mycosis)					
	Live larvae ^a		Dead larvae ^b		Dead adults ^c	
	Furrows	Hills	Furrows	Hills	Furrows	Hills
<i>B. bassiana</i>	1.16 \pm 0.66	0.24 \pm 0.07	15.48 \pm 1.12 (97.2%)	3.64 \pm 0.81 (97.8%)	0.16 \pm 0.08 (100%)	0.08 \pm 0.05 (100%)
<i>B. thuringiensis</i>	2.44 \pm 0.66	0.36 \pm 0.07	1.40 \pm 1.12 (82.9%)	0.52 \pm 0.81 (100%)	0.04 \pm 0.08 (100%)	0.04 \pm 0.05 (100%)

^aDensities of live larvae were significantly higher in furrows than on hills (F=11.8; df=1,4; P=0.03); larval densities were similar in fungus treated and *Bt*-treated plots (F=1.93; df=1,4; P=0.24); by repeated measures ANOVA; sampling date August 7, 1997.

^bNumbers of dead larvae were significantly higher in furrows than on hills (F=250.6; df=1,4; P<0.001); and were significantly higher in fungus-treated plots than in *Bt*-treated plots (F=42.2, df=1,4, P=0.003); by repeated measures ANOVA.

^cNumbers of dead adults were similar between furrow and hill locations (F=0.19; df=1,4; P=0.69); and were similar between fungus- and *Bt*-treated plots (F=4.6, df=1,4, P=0.1); by repeated measures ANOVA.

Beat tray samples: nontarget insect pests. Beat-tray counts were dominated by a subset of taxa (Figures 2 and 3). Winged aphids [primarily green peach aphid, *Myzus persicae* (Sulzer)], were abundant on 2 July but rapidly declined in numbers during following weeks (Figure 2). Densities of winged aphids were similar among the plots not treated with aldicarb except on July 16 when numbers were lower on the control plots due to severe defoliation (Figure 2). By this date, control plots had been extensively defoliated by CPB, and this may have reduced attractiveness of these plots to flying aphids. Densities of wingless aphids (primarily green peach aphid) peaked on July 16, and were not significantly different among plots not receiving aldicarb (Figure 2). Densities of adult Miridae (mostly *Lygus* spp.) were fairly low all season and were not affected by treatment. Numbers of immature Miridae peaked on July 31, and densities were highest in the plots treated with the high rate of *Bt* (Figure 2). Leafhopper and planthopper counts peaked in mid-August, and these insects also were most abundant in the plots treated with a high rate of *Bt*. Potato psyllid [*Paratrioza cockerelli* (Sulc)], an important pest of potato in the eastern United States growing regions, was encountered only very rarely in this study.

Beat tray samples: beneficial insects. Densities of adult *Geocoris* spp. peaked in mid-July with numbers approaching two insects/tray in plots that had been sprayed with the high rate of *Bt* (Figure 3). Nymphs of *Geocoris* spp. began appearing in late July, but numbers remained low (< 1/10 trays) despite the high

densities of adults on earlier sampling dates. The low numbers of *Geocoris* nymphs may have been correlated with the decline in aphid density from mid to late July (Figure 2). Numbers of *O. tristicolor* also were highest in the plots sprayed with the high rate of *Bt* (Figure 3). Nymphal *O. tristicolor* were rarely seen in the aldicarb-treated plots until mid-August (Figure 3). Other predatory Heteroptera occasionally seen on beating trays included damselbugs (Nabidae), *Anthocoris antevolens* White, *Deraeocoris brevis* Uhler, *Perillus bioculatus* (Fab.), and immature assassin bugs (Reduviidae). Predatory Heteroptera peaked in numbers in mid-July (Figure 3), with densities highest in the plots sprayed with the high rate of *Bt*.

Several other natural enemy taxa were seen in beating tray samples at very low densities, including Coccinellidae [maximum counts of 0.8/10 trays (July 16)], parasitic Hymenoptera [maximum 1.6/10 trays (July 16)], lacewings [Neuroptera; maximum 0.28/10 trays (July 31)], earwigs [Dermaptera; maximum 0.12/10 trays (July 31)], and Syrphidae [maximum 0.32/10 trays (July 31)]. A tachinid parasitoid [*Myiopharus doryphorae* (Riley)] associated with CPB causes quite high mortality of the beetle in the study area, but was not encountered due to the sampling method employed. Also, despite very high densities of the beetle, the predator *P. bioculatus* was only occasionally seen in the plots [maximum 0.24/10 trays (August 15)]. Similar densities of spiders were observed in all treatments in the first half of July. Surprisingly, the highest numbers of spiders in beat tray samples in late July and

Table 3. Average number of overwintering Colorado potato beetles/0.03 m³ (ft³) in plots receiving no intervention and in plots treated with the Mycotrol WP formulation of *Beauveria bassiana*, the Raven flowable formulation of *Bacillus thuringiensis* or aldicarb

Treatment	Mean no. ^a ± s.e. beetles/0.03 m ³				
	Live adults ^b	Total dead ^c	Mycosed adults ^d	Mycosed larvae ^e	Mean% ± s.e. mortality ^f
Control	3.44 ± 0.64 a	0.40 ± 0.21 b	0.24 ± 0.12	0 ± 0.15 b	14.1 ± 0.09 bc
<i>B. bassiana</i> (5 × 10 ¹³ spores ha ⁻¹)	0.84 ± 0.64 b	1.20 ± 0.21 a	0.04 ± 0.12	1.00 ± 0.15 a	56.7 ± 0.09 a
<i>B. thuringiensis</i> (1.17 l ha ⁻¹)	0.72 ± 0.64 b	0.60 ± 0.21 ab	0.40 ± 0.12	0.20 ± 0.15 b	42.5 ± 0.09 a
<i>B. thuringiensis</i> (7.04 l ha ⁻¹)	0.68 ± 0.64 b	0.36 ± 0.21 b	0.08 ± 0.12	0.16 ± 0.15 b	36.2 ± 0.09 ab
aldicarb (3.37 a.i. kg ha ⁻¹)	1.84 ± 0.64 ab	0.12 ± 0.21 b	0.04 ± 0.12	0 ± 0.15 b	9.3 ± 0.09 c

^aMeans within the same column followed by the same letter are not significantly different (LSD test; P<0.05).

^bF=3.43; df=4,16; P=0.03.

^cMycosed and nonmycosed adults & larvae. F=3.87; df=4,16; P=0.02.

^dF=1.85; df=4,16; P=0.17.

^eF=7.93; df=4,16; P<0.001.

^fF=4.97; df=4,16; P=0.009.

mid-August were seen in plots treated with aldicarb (Figure 3).

Pitfall samples. Arthropod numbers in pitfall traps were independent of treatment except in a few specific instances (Figure 4). Numbers of adult *Geocoris* spp. were very low in the aldicarb plots, similar to results noted with the beat tray samples. Moreover, early-season counts of *Geocoris* were significantly larger in plots treated with a high rate of *Bt* than in the remaining treatments (Figure 4), and this trend resembles that noted for predatory Heteroptera collected by beat trays (Figure 3). Ground beetles were extremely numerous in all plots, particularly in early July (Figure 4); samples were dominated (>90% of carabids) by a species that we have tentatively identified as belonging to the genus *Bembidion*. Counts of Carabidae were not affected by treatment. Pitfall samples of Staphylinidae were also independent of treatment, despite a suggestion that early-season numbers may have been lower in the aldicarb-treated plots than in the other plots (Figure 4; treatment effects non-significant). Counts of spiders in pitfall traps peaked in late-summer (Figure 4). Season-long counts of spiders were significantly higher in pitfall traps in the plots treated with the low rate of *Bt* than in the other treatments, for unknown reason.

Table 4. Average potato yield per 15.2 m row in plots receiving no intervention for Colorado potato beetle and those treated with the Mycotrol WP formulation of *Beauveria bassiana*, the Raven flowable formulation of *Bacillus thuringiensis* or aldicarb

Treatment	Mean yield ± s.e. (kg row ⁻¹) ^a	Metric tons ha ⁻¹
Control	31.54 ± 2.25 a	24.0
<i>B. bassiana</i> (5 × 10 ¹³ spores ha ⁻¹)	43.64 ± 2.25 b	33.2
<i>B. thuringiensis</i> (1.17 l ha ⁻¹)	43.64 ± 2.25 b	33.2
<i>B. thuringiensis</i> (7.04 l ha ⁻¹)	52.53 ± 2.25 c	40.0
aldicarb (3.37 kg a.i. ha ⁻¹)	59.88 ± 2.25 d	45.6

^aMean yields followed by the same letter are not significantly different from one another as determined with the LSD test and ANOVA (F=22.53, df=4,16 P<0.001).

Discussion

Numerous field tests have been conducted with *B. bassiana* and *B. thuringiensis* against CPB in Europe and North America. Efficacy of *B. bassiana* for control of CPB has ranged from mediocre (Fargues et al., 1980; Hajek et al., 1987) to good (Hajek et al., 1987; Poprawski et al., 1997). In some cases, control comparable to that obtained with some broad spectrum insecticides has been observed (Poprawski

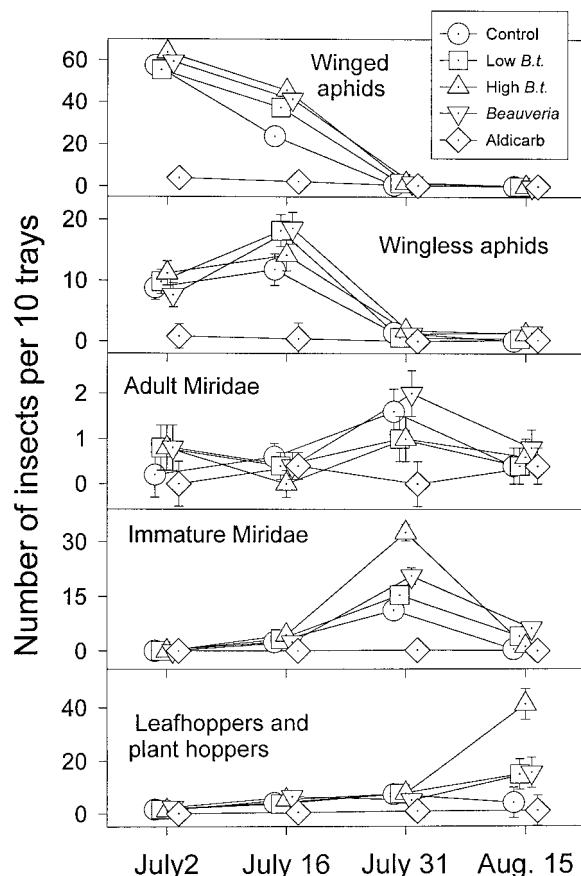


Figure 2. Mean (\pm s.e.) numbers of aphids, Miridae, and leafhoppers per 10 trays in experimental plots. Counts compared among treatments by one-way ANOVA conducted separately for each date. Least significant differences for sample dates on which the ANOVA was significant are: winged aphids (2 July and 16 July, respectively) 12.1 and 6.5; wingless aphids (2 July to 31 July, respectively) 6.1, 7.9, and 1.2; adult Miridae (no treatment effects); immature Miridae (16 July to 15 August, respectively) 1.7, 6.9, and 3.1; leafhoppers (31 July and 15 August, respectively) 2.4 and 17.3.

et al., 1997). Experimental and commercial formulations of *Bt* with beetle-active toxins (*cry* 3A and 3Bb) have produced excellent control of CPB (Langenbruch et al., 1985; Ferro & Gelernter, 1989; Zehnder & Gelernter, 1989; Ghidui & Zehnder, 1993). The suppression of CPB, reduction of defoliation, and tuber yields obtained in our plots that were treated with *B. bassiana* and *Bt* were similar to those reported by several other researchers when successful control was achieved with microbials.

The main limitation of both microbial agents, particularly as compared to the long-duration effects of aldicarb, is short residual activity necessitating several treatments. Ferro et al. (1993) reported rapid decline

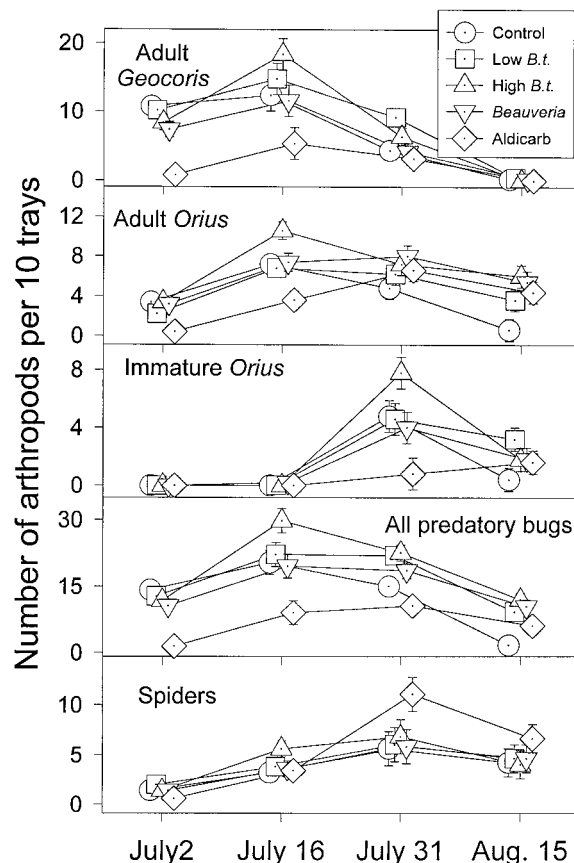


Figure 3. Mean (\pm s.e.) numbers of *Geocoris* spp., *Orius tristicolor*, total predatory Heteroptera, and spiders (Araneae) per 10 trays in experimental plots (results for predatory Heteroptera exclude taxa not known to feed on aphids [e.g., *Perillus bioculatus*]). Counts compared among treatments by one-way ANOVA conducted separately for each date. Least significant differences for sample dates on which the ANOVA was significant are: adult *Geocoris* spp. (2 July to 31 July, respectively) 3.3, 7.0, and 3.6; adult *Orius tristicolor* (2 July, 16 July, and 15 August, respectively) 1.9, 2.6, and 3.4; immature *O. tristicolor* (31 July) 3.2; predatory Heteroptera (2 July to 15 August, respectively) 3.8, 8.0, 5.3, and 4.6; spiders (no treatment effects).

in larvicidal activity of *Bt* under field conditions. The detrimental effects of ultraviolet (UV) light on spore viability of *B. bassiana* and other fungi has been well documented (Fargues et al., 1996). By applying microbials in early evening to minimize solar deactivation and also eliminating overhead irrigation for 24 h after application in this study presumably prolonged exposure of CPB to the entomopathogens. Formulation components that block UV light should extend the activity further.

Factors that affect activity of both agents against CPB include age of larvae, dosage, and temperature

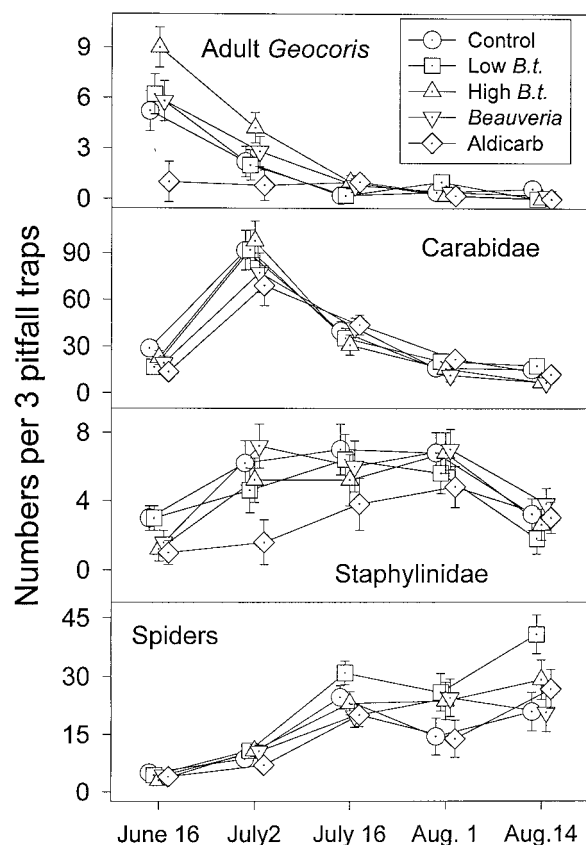


Figure 4. Mean (\pm s.e.) numbers of adult *Geocoris* spp., Carabidae, Staphylinidae, and spiders collected per 3 pitfall traps in experimental plots. Counts of *Geocoris* compared among treatments by one-way ANOVA conducted separately for each date. The least significant difference for the only sample date (16 June) on which the ANOVA was significant is 3.5 *Geocoris* per 3 traps. Treatment effects were non-significant for Carabidae and Staphylinidae. Season-long counts of spiders were affected by treatment (repeated measures ANOVA: $F=5.77$; $df=4,16$; $P<0.005$ [treatment \times date interaction non-significant]).

(Fargues, 1972; Zehnder & Gelernter, 1989; Ferro & Lyon, 1991; Fargues et al., 1994). Humidity, biotype of *B. bassiana* and solar radiation affects activity of the fungus (Fargues, 1972). Fargues (1972) observed that there was no difference in the susceptibility of CPB instars to the fungus if total mortality was taken into account, but the delay in death seen in older instars was significant. Because large larvae produce most of the damage to plants, a delay in mortality could result in significant defoliation.

Fargues et al. (1994) reported reduced leaf consumption by still living infected larvae. Despite the presence in *B. bassiana* treated plots of 10 small and 12 large larvae/plant in the July 15 samples and 10

large larvae/plant in the July 29 samples, some recovery of foliage was apparent in early August relative to defoliation scores made July 22. The recovery could be due in part to reduced feeding or lack of feeding by living, but infected larvae.

Another benefit of *B. bassiana* infections is the production of secondary inoculum. Conidia produced in cadavers on the soil surface increased the chances of uninfected larvae coming into contact with the fungus *en route* to pupation sites in the soil. Significantly more mycosed larvae in the soil of the *Beauveria*-treated plots in our study is an indication of continued entomopathogenic activity in larvae that entered the soil for pupation.

One of the major obstacles for using entomopathogenic fungi in desert environments is the requirement for high humidity. Very little control of larvae was apparent in *Beauveria*-treated plots until row closure, ostensibly coinciding with higher humidity and increased protection from sunlight. Despite the lateness in the season and presence of later instars, greater control in the second half of the sampling period was obtained than that observed following the first two applications.

Poprawski et al. (1997) recommended applying *B. bassiana* early in the season when eggs and younger larvae are present and to make four applications at shorter intervals than that used in our study. However, in the conditions of our study, application of *B. bassiana* before row closure, as would be the case if the applications had been made earlier and at four day intervals, probably would have been less effective in desert conditions.

Older larvae appear to be less susceptible to *Bt* than do first and second instars. Zehnder & Gelernter (1989) noted a greater extent of recovery in third instars and adults than second instars following exposure to the bacterium. Several researchers have recommended that applications of *Bt* be timed to coincide with CPB egg hatch or when early instars predominate (Ferro & Gelernter, 1989; Zehnder & Gelernter, 1989; Zehnder et al., 1992; Ghidiu & Zehnder, 1993). Although our initial applications were made when larvae were small and eggs were still present, the subsequent 4 sprays were made when older larvae were present. Some suppression of older larvae was evident in plots treated with the low rate of the bacterium, following the last two applications. This effect could have been due to retardation of development or mortality of younger larvae or actual mortality in older instars. Cloutier & Jean (1998) reported that large larvae sur-

living sublethal dosage of *Bt* stop feeding briefly, but soon resume at a relatively slow rate. Plots treated at the high rate were virtually devoid of large larvae.

Despite heavy defoliation in the plots treated with the low rate of *Bt* late in the growing season, yields of tubers were significantly greater than that of the control plots and identical to that in plots treated with *B. bassiana*. Hare (1980) reported that early and late season defoliation were not correlated with yield, but damage which occurred in the middle 4–6 weeks of the season resulted in reduced yield.

The contrast between plots treated with entomopathogens and those treated with the systemic insecticide aldicarb in terms of effects on nontarget organisms is striking. A rich diversity of predatory arthropods is known to inhabit potato fields (Boiteau, 1983; Hough-Goldstein et al., 1993; Ferro, 1994; Hilbeck & Kennedy, 1996). Our data from control and microbial-treated plots corroborate these observations. Perhaps the most noticeable consequence of the early-season treatment with the systemic insecticide was that the deleterious effects on densities of natural enemies were apparent for the entire growing season. This response appears to differ from that caused by other types of chemical insecticides in potato, in which treatment may cause an immediate, but temporary decline in predator numbers (Hilbeck & Kennedy, 1996). Some of the season-long effect on predators noted in this study was certainly due to a lack of aphid, leafhopper, or mirid prey in the aldicarb plots. However, for at least some predators, the systemic nature of aldicarb may also have been harmful. Many species of predatory Heteroptera feed to some extent on plants, including several taxa seen in this study (Stoner, 1970, 1972), and these insects may have been harmed directly by the pesticide. Anthocorid predators have been shown to take up systemic insecticides from plants during feeding activities, with subsequent increases in mortality occurring in some instances (Elliott, 1970). Moreover, many predatory Heteroptera insert their eggs directly into plant tissues where they may be susceptible to systemic pesticides (Elliott & Way, 1968). Virtually no immature Heteroptera were seen in the aldicarb-treated plots.

Despite the presence of natural enemies in the control and microbial plots, it remains unclear whether these predators assisted with significant biocontrol of pests. Microbial control agents and predators of CPB appear to be compatible, as shown by researchers in the eastern United States and Canada (Poprawski et al., 1997; Hough-Goldstein & Keil, 1991; Cloutier

et al., 1995; Cloutier & Jean, 1998), although it often is unclear whether the compatibility actually translates into enhanced control of the beetle (e.g., Cantwell et al., 1985; Poprawski et al., 1997). In this study, we observed predation on larval CPB by several predatory Heteroptera, including *P. bioculatus*, Nabidae, and Reduviidae. We also assume that eggs of the beetle were fed upon by several of the common predatory taxa recorded on trays or in pitfalls (Hilbeck & Kennedy, 1996). The most important invertebrate enemy of the beetle in the study area may actually be a tachinid parasitoid, *M. doryphorae*, rather than a predatory species (Tamaki et al., 1983). The effects of the different treatments on this species are unknown, as we did not collect the fly on our beat-tray samples nor did we sample beetle larvae to determine parasitism rates.

Other predatory taxa appeared not to show a numerical decline in the aldicarb-treated plots, most notably ground beetles (Carabidae) and spiders. Some taxa of spiders appear to readily accept CPB larvae as prey and may thus be important predators in potato (Hilbeck & Kennedy, 1996). Carabid beetles are often very abundant in potato fields (Boiteau, 1983) and have been shown to be important predators of immature CPB (Sorokin, 1981; Ferro, 1994). The importance of carabids in reducing beetle numbers in this study is unknown. Pitfall collections of carabids were dominated by *Bembidion*, a genus that is common in certain potato growing regions (Boiteau, 1983; Sorokin, 1981). The beetle was extremely abundant in all plots of this study. We did not collect this ground beetle on beat trays, which may indicate either that the insect does not colonize the potato plant or that it is mostly nocturnal.

The efficacy of both *B. thuringiensis* and *B. bassiana* in the irrigated potato agroecosystem, the presence of the fungus in CPB in subsoil habitats and compatibility of the two microbials with insect natural enemies is encouraging. However, the potential for replacing broad-spectrum insecticides with microbial insecticides to control CPB in certain growing regions may actually depend primarily upon pests other than CPB. In the Pacific Northwest, the most damaging insect pest of potatoes is the green peach aphid, because it is the primary vector of potato leaf roll virus in the area. Only extremely low densities of the aphid can be tolerated, and insect pest management in the Northwest revolves around controlling this species, not CPB (which is incidentally controlled by products directed at the aphid). Thus, a biological control program directed at CPB would be accepted by grow-

ers only if aphid-control was not negatively affected. In the present study, wingless aphids never became abundant in any of the plots, even though densities of migrants (winged aphids) were fairly high. This observation suggests that natural enemies were active in the microbial-treated plots. It is unknown, however, whether the densities observed in the microbial-treated plots were sufficiently low to prevent economic damage due to leaf roll virus. Considerably more research is necessary to determine the manner in which microbial control of CPB affects the aphid-potato interaction, particularly in the growing regions of western North America.

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